REMARKS

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

The specification is being amended to add sequence identifier numbers, as requested by the examiner. A substitute sequence listing is also being submitted herewith, which includes the sequences corresponding to the newly added sequence identifiers. No new matter is being added.

Claims 38, 39, 41, 57, and 58 are currently being amended. These amendments make minor grammatical changes suggested by the Examiner and remove a phrase previously added and deemed objectionable by the Examiner. In addition, claim 38 is amended to clarify that the cell-free medium results from culturing of the PTH production host. No new matter is being added.

A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

It is acknowledged that the claim amendments are submitted after final rejection of the claims. However, because the claim amendments do not introduce new matter, and either place the application in condition for allowance or at least in better condition for appeal, entry thereof by the Examiner is respectfully requested. After amending the claims as set forth above, claims 38, 39, 41, and 57-59 are now pending and being examined on the merits.

I. Formal Matters

Applicants note the formal matters presented by the Examiner. The SB/08 form accompanying the Information Disclosure Statement filed on October 28, 2004, should only be one page and list eight references. The notation at the top of the SB/08 form indicating that two sheets were being submitted is a typographical error. Applicants appreciate the Examiner noting this discrepancy.

II. Specification

The Examiner objects to the specification because "Applicants have not provided Sequence Identifiers (SEQ ID NOs) for the signal sequence disclosed on page 25, lines 10, 18, and 26." Office action at 2.

The sequences on page 25, lines 10, 18, and 26 are accompanied by appropriate sequence identifiers, as amended. Thus, Applicants respectfully request withdrawal of this ground of rejection.

III. Claim Objections

The Examiner objects to claims 37, 38, 41, and 57-59 because the term "cell free" should be hyphenated. Claim 37 has been cancelled, and Applicants have amended claims 38, 39, 41, 57, and 58 to recite "cell-free." Accordingly, Applicants respectfully request withdrawal of this ground of objection.

IV. Double Patenting

Applicants note and appreciate the Examiner holding in abeyance the double patenting rejection over co-pending U.S. Application No. 08/340,664 until the present application is in condition for allowance.

V. Claim Rejections – 35 U.S.C. § 112, first paragraph - Enablement

The Examiner has withdrawn the rejection of claims 38, 39, 41, and 57-59 under 35 U.S.C. § 112, first paragraph, because "the claims are drawn toward 'medium' and not towards methods of making the PTH protein." Office action at 2. Applicants agree that the present claims are enabled. However, Applicants do not acquiesce to the conclusion that hypothetical claims to "methods of making PTH protein" would not be enabled.

VI. Claim Rejections – 35 U.S.C. § 112, first paragraph – Written Description

Claims 38, 39, and 41 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. Specifically, the Examiner rejects the claims for the recitation "said PTH fraction does not contain chemically modified amino acids" in claim 38.

While not acquiescing in the propriety of the rejection, Applicants have amended the claims to remove the recitation "said PTH fraction does not contain chemically modified amino acids" from claim 38. Thus, the rejection is rendered moot.

VII. Claim Rejections – 35 U.S.C. § 112, second paragraph – Definiteness

Claims 38, 39, 41, and 57-59 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite based on the term "chemically modified." While not acquiescing in the propriety of the rejection, the claims no longer recite the phrase "chemically modified," as amended. Thus, the rejection is rendered moot.

VIII. Claim Rejections – 35 U.S.C. § 102 – Anticipation

A. Breyel *et al.*, THIRD EUROPEAN CONGRESS ON BIOTECHNOLOGY 3:363-369 (1984)

Claims 38, 39, 41, and 57-59 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Breyel *et al.*, *Synthesis of mature human parathyroid hormone in Escherichia coli*, THIRD EUROPEAN CONGRESS ON BIOTECHNOLOGY 3:363-369 (1984) ("Breyel"). Applicants respectfully traverse this ground of rejection.

The claimed invention is novel and non-obvious over Breyel, as discussed in Applicants' Amendment of December 31, 2003. The expression product of the Breyel system accumulates only intracellularly. This is because the Breyel system is not a secretion system that would enable the expression product to accumulate outside the cell. Instead, Breyel's system is designed to retain PTH within the host, where it remains subject to the action of endogenous proteases. The consequences of using the Breyel production system are thus at least two-fold: (1) on the one hand, any PTH produced using the Breyel system must be

extracted by first lysing the host cells, and (2) on the other hand, any PTH available for extraction from the host cells is degraded before it can be extracted. Accordingly, Breyel's PTH fraction does not "consist[] essentially of intact hPTH(1-84) molecules," as required by Applicants' claims.

In contrast, the cell free medium of the claimed invention "consists essentially of intact hPTH(1-84) molecules." The cell-free medium of the claimed invention is not obtained through cell lysis; rather, the PTH is secreted into the medium by the microorganism. Because the cell-free medium of the claimed invention is not obtained by lysing the cells, protein degradation of the PTH by proteases present in the cell interior is minimal.

1. Breyel's PTH is not secreted from the cells into the culture medium

The Examiner argues that: (a) Breyel's "discarded medium, which would have been discarded before the cells were lysed, would have contained intact PTH", and (b) "PTH, which is normally secreted into the medium, would be present, undegraded, in the 49 ml of 'unused' culture." Office action at 3 (emphasis added).

However, both of these arguments are based on an incorrect factual assumption – that Breyel's PTH is secreted from cells. In fact, Breyel's PTH is not secreted from the cells and can only be obtained by breaking the cells open, *i.e.*, lysing the cells. Breyel at 364. Indeed, Breyel's system is characterized later as an intracellular production system, by Hogset *et al*, BIOCHEM. BIOPHYS. RES. COMMUN. 166(1):50-60 (1990) (Exhibit A) (see directly under "Discussion" section) who review the type and capabilities of prior art PTH production systems, including that of Breyel. Moreover, the Board of Patent Appeals and Interferences, in their decision in the present application's parent case (U.S. Application No. 08/340,664), saw fit to comment that "there is no suggestion in ... Breyel ... that any intact protein could be obtained", a statement that applies regardless of whether the product might be located in the medium (which it is not), or within the Breyel cells. Board decision mailed July 2, 2003, page 9 (Exhibit B) (emphasis added). Thus, Breyel's medium does not contain PTH(1-84) secreted from cells.

2. Breyel's PTH is degraded by proteases

The Examiner argues that "[r]egardless of the fact that the cells of Breyel were lysed, this does not mean that PTH was degraded". Office action at 3. This statement is factually incorrect as evinced by Breyel. Indeed, Breyel notes that PTH degradation occurred rapidly after expression. Breyel at 366, 367 (Fig. 3). Thus, the PTH of Breyel does not "consist[] essentially of intact hPTH(1-84) molecules." Moreover, as stated by the BPAI, "there is no suggestion in Breyel that any intact protein could be obtained". Neither is there any reason, given the design of the Breyel production system, to expect that intact PTH could be, or would be, located in the spent culturing medium, as presently claimed.

3. Breyel does not teach or suggest the claimed invention

For the reasons discussed above, the cell free medium of the claimed invention is not anticipated by the extract of Breyel and, therefore, withdrawal of this ground for rejection is respectfully requested.

B. Applicants' Specification – PTH Standard

Claims 38, 39, 41, and 57-59 stand rejected under 35 U.S.C. § 102 as anticipated by the PTH standard mentioned in Applicants' specification. Specifically, the Examiner agrees that "[t]he [Gautvik] Declaration is persuasive in demonstrating that the PTH of the invention is different from that of the standard." Office action at 4. However, the Examiner maintains the rejection "since the specification has not provided a definition of the phrase 'chemically modified.'" *Id.*

While not acquiescing in the propriety of the rejection, the claims have been amended to no longer recite "chemically modified." Thus, this ground of rejection is rendered moot.

IX. Claim Rejections – 35 U.S.C. § 103

Claims 38, 39, 41, and 57-59 stand rejected under 35 U.S.C. § 103(a) as being allegedly obvious over Breyel or Mayer *et al.* (EP 0 139 076) ("Mayer") in view of Kaisha *et*

al. (GB 2 092 596) ("Kaisha") and Brewer *et al.* (U.S. Patent No. 3,886,132) ("Brewer"). Applicants respectfully traverse this ground of rejection.

The claimed invention is non-obvious over Breyel or Mayer in view of Kaisha and Brewer, as discussed in Applicants' Amendment of October 28, 2004. One of ordinary skill in the art would know that the purification process of Brewer could not by applied to either Breyel or Mayer, because the PTH of Breyel and Mayer were derived from entirely different sources than the PTH of Brewer. Thus, one of ordinary skill in the art would have no motivation to use the process of Brewer to purify the PTH of either Breyel or Mayer. Simply, neither Breyel nor Mayer teach the production of the claimed cell-free medium, and thus both references fail to provide such a source of PTH for any application of the Kaisha purification system.

Accordingly, the Examiner has failed to establish a prima facie case of obviousness. Withdrawal of this ground for rejection is respectfully requested.

A. Neither Breyel nor Mayer teach or suggest a cell-free medium that "consists essentially of intact hPTH(1-84) molecules"

Claims are given their broadest reasonable interpretation consistent with the specification during examination. MPEP § 2111. In reaching the broadest reasonable interpretation, "the claims are not to be read in a vacuum." *Id.* (quoting *In re Marosi*, 710 F.2d 799 (Fed. Cir. 1983)). Instead, the claims "are to be interpreted in light of the specification in giving them their 'broadest reasonable interpretation'." *Id.* (emphasis original).

The Examiner alleges that the PTH fractions of Breyel and Mayer meet the present claim limitations absent a definition of "consists essentially of intact hPTH(1-84) molecules." However, defining "consists essentially of intact hPTH(1-84) molecules" to encompass the PTH fractions of Breyel and Mayer is clearly unreasonable in light of the specification.

The specification repeatedly warns of the degradation problems associated with PTH expression encountered using conventional techniques. *See e.g.*, Application at 16, lines 17-

25. Specifically, "hPTH is an easily degraded polypeptide," and conventional expression systems produced degraded, *i.e.*, fragmented, PTH with reduced biological activity. *Id.* Indeed, Applicants addressed Breyel directly and noted that Breyel "demonstrated that E. coli degrades human PTH." Application at page 3, lines 3-6. Thus, Applicants repeatedly distinguished their methods of producing intact hPTH from conventional systems, which resulted in degraded PTH. *See e.g.*, Application at 7, lines 12-35.

In light of the specification's teachings, one of skill in the art would conclude that the inclusion of fragmented PTH found in the prior art in the claimed cell-free medium would "'materially affect the basic and novel characteristic(s)' of the claimed invention." MPEP § 2111.03 (quoting *In re Herz*, 537 F.2d 549, 551-52 (CCPA 1976)). Accordingly, the extracts of Breyel and Mayer are not encompassed by a reasonable construction of "said PTH fraction consists essentially of intact hPTH(1-84) molecules," because the PTH of both Breyel and Mayer is fragmented. *See* MPEP § 2111.03. A contrary construction, which includes the extracts of Breyel and Mayer, would improperly ignore the teachings of the specification and would therefore be unreasonable. *See* MPEP 2111.

B. The claimed cell-free medium does not necessarily contain endopeptidases and exopeptidases

The Examiner argues that the "cell-free medium would contain [endopeptidases and exopeptidases] in the absence of any method step to eliminate them." Office action at 4. Thus, the Examiner argues that there is an enablement "concern," because the peptidases would degrade the PTH.

On the contrary, the presence of any such enzymes in the claimed cell-free medium would be minimal. Clearly, they did not preclude the isolation of intact PTH as demonstrated in the present specification., a feat that was never accomplished by Breyel and is not possible using the Breyel system. For the reasons discussed above, the cited references do not teach or suggest the claimed invention and, therefore, withdrawal of this ground for rejection is respectfully requested.

CONCLUSION

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 C.F.R. § 1.136 and authorize payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date May 12, 2005

By Michele M Simkin

FOLEY & LARDNER LLP
Washington Harbour
3000 K Street, N.W., Suite 500
Washington, D.C. 20007-5143
Telephone: (202) 672-5538
Facsimile: (202) 672-5399

Michele M. Simkin
Attorney for Applicants
Registration No. 34,717

Vol. 166, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1990 Pages 50-60

EXPRESSION OF HUMAN PARATHYROID HORMONE IN ESCHERICHIA COLI

A. Høgset, O.R. Blingsmo, V.T. Gautvik, O. Sæther, P.B. Jacobsen, J.O. Gordeladze, *P. Alestrøm and K.M. Gautvik

Institute of Medical Biochemistry, University of Oslo, p.o. box 1112 Blindern, 0317 Oslo 3, Norway

*Agricultural University of Norway, p.o.box 36, N-1432 Ås-NLH, Norway

Received June 28, 1989

Human parathyroid hormone (hPTH) is a peptide hormone consisting of 84 amino acids. Using the expression plasmid pKK223-3 with the strong tac-promoter, we have produced a variant of hPTH in E. coli. From the expression plasmid construct the expected product was hPTH with an N-terminal extension of Met-Gly. The peptide was extracted from E. coli cells and purified by high performance liquid chromatography. In two different gel electrophoresis systems including identification by immunoblotting the product behaved exactly as an hPTH standard. N-terminal amino acid sequence analysis of the purified product showed traces of Gly-hPTH. At least 90% of the expressed product was N-terminally blocked, suggesting the presence of N-formyl-methionine. This variant of hPTH did not stimulate adenylate cyclase activity in rat osteosarcoma cell membranes.

Human parathyroid hormone (hPTH) is an 84 amino acid peptide which is secreted from the parathyroid glands. The primary translation product is a 115 amino acid preprohormone, and the prepro part is cleaved off during secretion, yielding the 84 amino acid mature hormone (1).

hPTH is a principal homeostatic regulator of blood calcium and phosphate through its actions on kidney and bone (2,3). Prolonged exposure to low doses of an N-terminal fragment of PTH stimulates bone formation in vivo (4,5), while chronic overproduction (hyperparathyroidism) leads to demineralization.

Abbreviations: PTH, parathyroid hormone; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; PBS, phosphate buffered saline (0.15 M NaCl, 0.015 M NaH_2PO_4, pH 7.4); IPTG, isopropyl-ß-D-thiogalactoside; fMet, N-formyl-methionine.
hormone, we have cloned its cDNA for expression in E. coli.

0006-291X/90 $1.50

The different domains within the PTH molecule appear to be specialized with respect to conveying its biological action in bone and kidney (2).

Therefore in order to learn more about the structure-function relationships of hPTH, and also to be able to do physiological studies with the intact hormone, we have cloned its cDNA for expression in E. coli.

MATERIALS AND METHODS

Strains, media and growth of cells

E. coli strains used were BJ5183 (6) and JM 103 (7). LB-medium contains 5 g Bacto Yeast Extract, 10 g Bacto Tryptone and 10 g NaCl per l, if necessary supplemented with 50 mg/l of ampicillin. For expression of hPTH, JM103 was grown in LB-medium with ampicillin. At an A_{600} of 0.2, IPTG (Pharmacia) was added to a final concentration of 1 mM.

Materials

Restriction enzymes, other DNA-modifying enzymes and [^{125}I]-antirabbit-IgG were from Amersham. The N-terminal-specific anti-PTH antibody was from Chemicon, the preparation of the other antibodies has been described earlier (8). Synthetic hPTH(1-84) was from Sigma or Bacham.

Cloning of hPTH cDNA

Poly(A) selected RNA was isolated from human parathyroid adenomas immediately after surgery. cDNA was prepared and cloned into the PstI site of pBR322 by the GC-tailing method as described by Maniatis et al. (9), and E. coli-strain BJ5183 was transformed by the method of Hanahan (7). The library was screened with synthetic oligonucleotides, based on the published sequence of cDNAs for hPTH (10).

DNA sequencing was done according to the methods of Maxam and Gilbert (11) and Chen and Seeburg (12).

Radioimmunoassay

Radioimmunoassay of hPTH was carried out as described (8) using a monospecific, polyvalent antiserum reactive against epitopes between amino acids 44 and 68 in hPTH.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis in the presence of SDS was performed as described by Laemmli. (13). Gels were silver stained with a BioRad silver staining kit according to the manufacturers recommendations.

For electrophoresis in the presence of acetic acid and urea, the gel was made up with a solution containing 4.5 M urea and 0.9 M HAc. Freeze-dried samples were dissolved in a sample buffer containing 0.9 M HAc, 8 M urea, 2% 2-mercaptoethanol and 0.05% pyronine Y.

Immunoblotting

Proteins fractionated by PAGE were transferred electrophoretically to Immobilon PVDF Transfer Membranes (Millipore), and staining of the filters and antibody was performed according to the manufacturers recommendations. Cock anti-PTH antiserum that reacts with epitopes within amino acid number 44-68 (8) was used (dilution 1:8000) as a primary antibody and anti-cock-IgG (dilution 1:1000) as the secondary antibody. As a tertiary antibody we used [^{125}I]-antirabbit-IgG from donkey. The N-terminal specific anti-hPTH rabbit antiserum was used (dilution 1:1000) with a secondary [^{125}I]-antirabbit-IgG from donkey.

Extraction of PTH from E. coli cells

Cells from 3 liters of culture were harvested by centrifugation at OD_{600}=1.0, and were washed 2 times in PBS containing 0.1 mM PMSF. The pellet was resuspended in 4 ml of lysozyme solution (10 mg/ml in water) and left at room temperature for 15 min. The wet weight of this suspension was determined, before being frozen at -70 °C. After thawing on ice, the suspension was sonicated 3 x 20 s with a Model W-10 Sonicator (Ultrasonics). PTH was then

extracted essentially as described by Aurbach (14). After ether precipitation and acetone washing (14) the dry powder was extracted with 20% acetone, 0.1% HAc, and the extracted material was used as the starting material for further purification. The yield of immunoreactive material at this stage was 200 µg per l of original culture, corresponding to a yield of about 20%.

HPLC-purification

PTH was further purified by reversed phase HPLC using a Vydac RP C18 protein/peptide column. A 25 cm x 4.6 mm column was used employing LDC constametric pumps model I and III, LDC gradient master, LDC spectromonitor III (LDC, Milton Roy Co, Riviera Beach, FL, USA) and a Vitatron 2 channel recorder. The experimental conditions were as follows: Eluants: A: 0.1% triflouroacetic acid in filtered and distilled H_2O; B: 70% acetonitrile in A. Flow: 1.0 ml/min. Gradient: 35-55% B (linear) in 48 min. Washing with 100% B for 10 min and equilibration with 35% B.

Amino acid sequence analysis

This was done on material separated by SDS-PAGE as described by Matsudaira (15) in a model 477A Protein Sequencer with an on-line 120A phenylthiohydantoin amino acid analyzer from Applied Biosystems (Foster City, CA, USA). All reagents were obtained from Applied Biosystems. Amino acid sequencing was performed by Dr. K. Sletten, Inst. of Biochemistry, University of Oslo.

Biological activity measurements

Recombinant hPTH was purified on HPLC and freeze dried. It was dissolved in distilled water and tested in the adenylate cyclase assay using of UMR 106 rat osteosarcoma cell membranes. Membranes were prepared and the assay carried out as previously described (16,17). The experiments was performed in triplicate determinations which differed by less than 17%.

RESULTS

Cloning and sequencing of cDNA for hPTH

A cDNA library was made as described in Materials and Methods. 66 out of 34 000 tested clones hybridized to the probes, and two of these were chosen for further study. DNA-sequencing showed that both clones contained the entire coding region for preproPTH, and that the sequence of the insert in clone 6 was identical to the sequence published by Hendy et al. (10). In clone 10 however, the sequence diverged from the published sequence in a position just upstream of the start codon (fig.1). This clone contained a double in-frame

```
hPTH cDNA (10)          TAT Gtg aag ATG ATA CCT
                                    Met Ile Pro

pSSHPTH-10                    T ATG ATG ATA CCT
                                Met Met Ile Pro

bPTH cDNA (22)                T ATG ATG tct gca
                                Met Met Ser Ala
```

Fig.1: The sequence of preproPTH cDNA from pSSHPTH10.
The plasmid pSSHPTH10 was purified by CsCl-centrifugation, and the insert was sequenced by the method of Maxam and Gilbert (11). The sequence round the translation start is compared to the sequences of cDNA for hPTH 10 and bovine PTH 22.

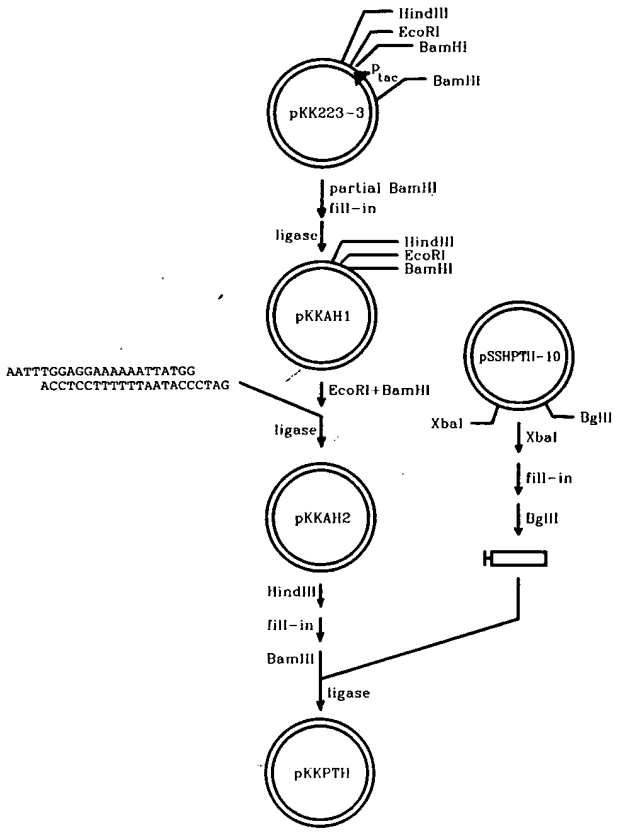


Fig.2: Construction of the hPTH expression plasmid pKKPTH.
For details, see text.

ATG at the beginning of the open reading frame, suggesting the possibility of alternative translation start-points. This sequence probably represented a 4 bases deletion from the sequence published by Hendy et al. (10), and was not found in other clones that were sequenced (data not shown). The plasmid containing this insert was designated pSSHPTH10.

Construction of an hPTH expression plasmid

pSSHPTH10 was used to construct an expression plasmid as outlined in fig.2. To destroy the BamHI-site outside the polylinker, the expression vector pKK223-3 (18) was partly digested with BamHI, and the sticky ends were filled in by the Klenow fragment of DNA polymerase I and religated with T4 DNA ligase. A vector-clone containing a unique BamHI-site in the polylinker was picked (pKKAH1), and a synthetic oligonucleotide containing a Shine-Dalgarno

sequence and an initiation codon was inserted between the EcoRI and BamHI sites to give the plasmid pKKAH2.

The plasmid pSSHPTH10 was digested with XbaI, filled in by Klenow polymerase and cut with BglII. The resulting 282 bp BglII-XbaI fragment was isolated and inserted between the BamHI and a blunt-ended HindIII site in pKKAH2 to give the final construct pKKPTH. In this construct the hPTH cDNA is under the transcriptional control of the IPTG-inducible **tac**-promoter (19) and the rrnb ribosomal RNA transcription terminator (20). This construct should express hPTH with a Met-Gly amino-terminal extension.

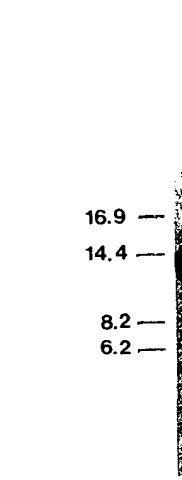
Expression of hPTH

For testing the expression level the cells were harvested at late log phase (A_{600}=1.0), disrupted by sonication, and radioimmunoassay was performed on both the soluble and the insoluble fraction after sonication. The expression level usually was about 2 mg PTH/l culture with more than 95% in the soluble fraction, indicating that Met-Gly-PTH does not aggregate in inclusion-bodies, in contrast to many other exogenous peptides produced in E.coli (21). The radioimmunoassay also showed that the PTH-related material was immunologically indistinguishable from hPTH (data not shown).

Analysis of the expression products by immunoblotting

Cell extracts were fractionated by SDS-PAGE and subjected to immunoblotting. As shown in fig.3 (lanes B and D) two PTH-immunoreactive peptides could be demonstrated in this experiment, and one of these co-migrated with the PTH-standard (lane C). This peptide could not be detected in extracts from control cells transformed with the vector alone (data not shown). The other band (approximately 14 kDa) corresponded to a dominant band seen when silver staining a part of the same gel (fig.3, lane A). The 14 kDa silverstained band probably mainly represents an E. coli protein, but as discussed below the antibody reactivity to this band probably is not due to unspecific binding, but reflects co-migration of a higher MW species of PTH made by E. coli.

A similar experiment was performed fractionating the proteins by HAc/urea PAGE. In this system the peptides are separated according to both charge and molecular weight. The major 14 kDa silverstained band seen after



(3)

Fig.3: Analysis of e
PTH expressi
IPTG. The cells we
(14), and subjected
were silver stained
reacted with antiseru
part (D) of hPTH.
Lane C was loaded
were loaded with
material as determin

Fig.4: Analysis of
phoresis and immu
Cell extracts
gel). Lanes were
filters and reacted w
terminal part (D,E)
rabbit-IgG. Lanes C
dard, lanes A,B an
ng (A) and 50 n
immunoassay.

SDS-PAGE (fig.3, la
urea PAGE (fig.4, la
with the PTH-standa

We also perfo
dimensional PAGE (d
dimension the band co
showing that the maj
standard in both dime
expression product, M

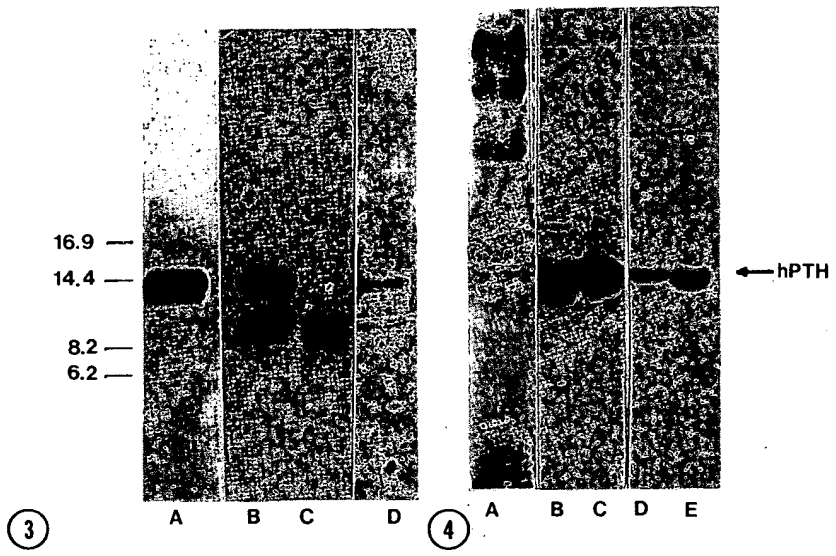


Fig.3: Analysis of expression products by SDS-PAGE and immunoblotting.
PTH expression in E.coli containing the plasmid pKKPTH was induced by IPTG. The cells were disrupted by sonication, and a cell extract was made (14), and subjected to SDS-PAGE (15% gel) under reducing conditions. Lanes were silver stained (A), or electroblotted onto nitrocellulose filters and reacted with antiserum against the mid-region part (B and C) or the N-terminal part (D) of hPTH. Antibody binding was detected by [^{125}I]-antirabbit-IgG. Lane C was loaded with 40 ng of hPTH (Sigma) as a standard, lanes A,B and D were loaded with E. coli extracts containing about 50 ng of hPTH-related material as determined by radioimmunoassay.

Fig.4: Analysis of expression products by HAc-urea polyacrylamide electrophoresis and immunoblotting.
Cell extracts (same as in fig.3) were subjected to HAc/urea PAGE (15% gel). Lanes were silver stained (A) or electroblotted onto nitrocellulose filters and reacted with antiserum against the mid-region part (B,C) or the N-terminal part (D,E) of hPTH. Antibody binding was detected by [^{125}I]-anti-rabbit-IgG. Lanes C and E were loaded with 40 ng of hPTH (Sigma) as a standard, lanes A,B and D were loaded with E. coli extracts containing about 500 ng (A) and 50 ng (B,D) of hPTH-related material as determined by radioimmunoassay.

SDS-PAGE (fig.3, lane A) apparently has split into several bands upon HAc-urea PAGE (fig.4, lane A), but the major immunoreactive band still co-migrated with the PTH-standard (fig.4, lanes B and D).

We also performed an experiment where the proteins were separated by two-dimensional PAGE (data not shown). After running HAc/urea PAGE in the first dimension the band co-migrating with the PTH-standard was run on SDS-PAGE, showing that the major immunoreactive peptide co-migrated with the PTH-standard in both dimensions. It therefore probably represents the expected expression product, Met-Gly-hPTH or a closely related molecular species.

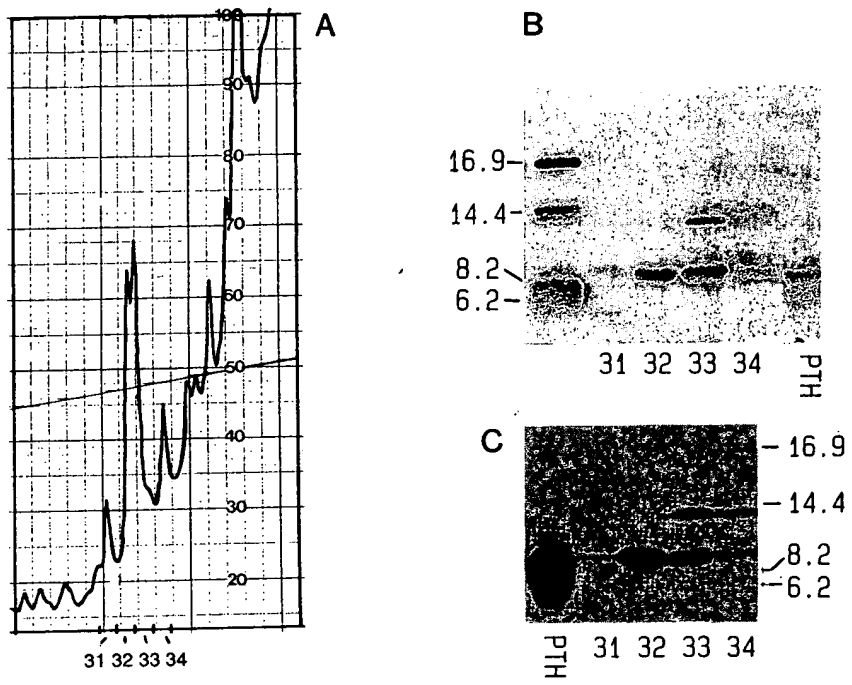


Fig.5: Purification of recombinant hPTH.
Met-Gly-PTH was purified from E. coli extracts by HPLC as described. PTH immunoreactive material in the fractions was detected by immunoblotting. (**A**) Elution profile from the HPLC purification, the fractions containing hPTH immunoreactivity are indicated. (**B**) SDS-PAGE, blotting and Coomassie-staining of the fractions indicated in (A). The fraction numbers are indicated under each lane. (**C**) Immunoblotting of the fractions from panel B using the mid-region antibody (except for the hPTH standard 10 times less material was loaded on the gel in panel C compared to panel B). Molecular weight standards are indicated, 1.5 µg hPTH(1-84) (Sigma) was loaded as a standard.

Purification and amino acid sequencing

The PTH-enriched cell extract was subjected to reverse-phase HPLC as described, and PTH in the fractions was detected by immunoblotting using the middle-region specific antibody. As shown in fig.5 (A and C) PTH-immuno-reactive material could be localized to a peak eluting with a retention time of 32-33 min. On SDS-PAGE and Coomassie-staining this peak was shown to consist of to major components (fig. 5B, fractions 32 and 33). One of these had an MW of about 9.5 kDa and co-migrated with the PTH-standard, the other component had an MW of about 14 kDa. Both of these proteins reacted with the anti-PTH antibody on immunoblotting, while the major 14 kDa polypeptide seen on SDS-PAGE (fig.3, lane A) eluted in the large peak appearing after the elution of PTH (fig.5 A) and did not react with the antibody (data not shown).

This indicates that the antibody reactivity to the 14 kDa protein (see also fig.3, lanes B and D) is due to this protein being PTH-related, and is not caused by unspecific binding to the major 14 kDa protein seen in fig.3 (lane A).

We then performed N-terminal amino acid sequence analysis on the peptide from fraction 32 (fig.5) that co-migrated with the PTH-standard. The sequence analysis gave a very low yield (much less than 10%), indicating that a large fraction of the sample was N-terminally blocked. A probable explanation for this is that fMet-hPTH could be a major constituent of this preparation. However a fraction of the preparation seemed to start with the amino acids X-Ser-Val-Ser-X-Ile which corresponds to the sequence expected if the N-terminal f-methionine residue has been removed to yield Gly-PTH.

Biological activity

PTH-related peptides from fraction 32 (fig.5A) were purified by HPLC as described, and biological activity was tested in the adenylate cyclase assay (16,17). This PTH preparation had no biological activity in concentrations that gave maximal stimulation of the enzyme activity with standard PTH (fig.6).

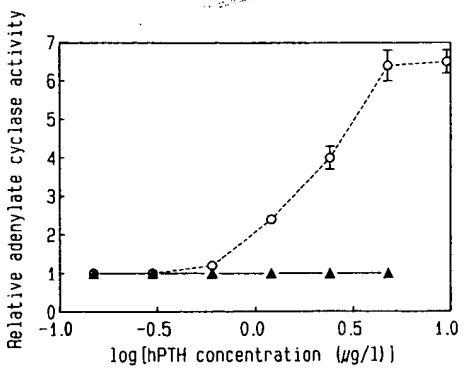


Fig.6: Biological activity of the purified recombinant hPTH.
Material from fraction 32 (fig.5) was tested in the adenylate cyclase assay as described in Materials and methods. The concentrations of hPTH was determined by radioimmunoassay. (○ ○) hPTH(1-84) standard, (▲ ▲) recombinant PTH. Standard deviations are indicated. The results are given as the mean of 3 determinations ± standard deviation.

DISCUSSION

The hPTH cDNA-clone used in this study had a sequence different from that previously published by Hendy et al. (10). In our clone 10 there were two consecutive methionine codons at the start of preproPTH instead of one as reported by Hendy et al. (10) (fig.1). This opens the possibility for an alternative translation start of preproPTH, in that the synthesis of Met-preproPTH might occur in addition to the synthesis of preproPTH. It is interesting to note that bovine preproPTH starts with two consecutive methionine residues (22). The significance of the observed sequence difference is unclear, and is currently under investigation in our laboratory.

This report describes the successful expression of slightly modified forms of human parathyroid hormone in E.coli. In immunoblotting experiments the PTH reactive material migrated as a sharp band, and no low molecular weight degradation products could be detected. Hence in our system hPTH apparently was quite stable, in contrast to what was reported by Breyel et al. (23) and Rabbani et al. (24). Compared to these reports we achieved about ten times higher production of hPTH, despite using the same promoter as Breyel et al. (23). A possible explanation for this might be that fMet-Gly-PTH is more stable in E. coli than fMet-PTH which was the major product expected from the constructs of these authors. Very recently Wingender et al. (25) reported high expression of a cro-ß-galactosidase-hPTH fusion protein which after acidic cleavage results in amounts of Pro-hPTH equivalent with ours.

Rabbani et al. (24) reported that the N-terminal methionine was partly cleaved off, yielding a small amount of correctly processed hPTH(1-84). Our data indicate that also when PTH is expressed as Met-Gly-PTH, E. coli is unable to deformylate effectively the initiator fMet. However the N-terminal methionine is apparently rapidly removed from deformylated molecules.

Born et al. (26) showed that the expression of preproPTH in E. coli directed the protein to the surface of the bacterial inner membrane, but that it was not properly processed. The major processed forms were PTH(3-84) and PTH(8-84). In our system we, however, find no indications of production of

hPTH(3-84) or PTH(8-84)as reported by Rabbani et al. (24) and Born et al. (26).

The hPTH-species produced in this work did not stimulate adenylate cyclase in our biological assay system, and hence apparently had no PTH-agonistic activity. The reason for this probably is that the amino terminal end is very important for the biological activity of PTH (2). Few studies have been done with amino terminal extensions of PTH, but addition of tyrosine at position -1, or Tyr-Gly-Gly at positions -3 through -1 caused a marked decline in biological activity (27). Moreover fMet-hPTH has only 10% of the activity of hPTH(1-84) (24). This calls for methods to produce high yields of hPTH with the correct N-terminal sequence. Such studies are in progress in our laboratory.

REFERENCES

1. Cohn, D.V., and MacGregor, R.R. (1981) Endocrine Rev. 2, 1-26.

2. Potts, J.T.Jr., Kronenberg, H.M., and Rosenblatt, M. (1982) Adv.Protein Chem. 35, 323-396.

3. Rosenblatt, M. (1984) Parathyroid hormone: intracellular transport, secretion, and receptor interaction (M.T.W. Hearn, ed.), pp.209-296. Marcel Dekker, New York.

4. Reeve, J., Meunier, P.J., Parsons, J.A., Bernat, M., Bijvoet, O.L.M., Courpron, P., Edouard, C., Klenerman, L., Neer, R.M., Renier, J.C., Slovik, D.M., Vismans, F.J.F.E., and Potts, J.T.Jr. (1980) Br.Med.J. 280, 1340-1344.

5. Slovik, D.M., Rosenthal, D.I., Doppelt, S.H., Potts, J.T.Jr., Daly, M.A., Campbell, J.A., and Neer, R.M. (1986) J.Bone Min.Res. 1, 377.

6. Losson, R., and Lacroute, F. (1981) Mol.Gen.Genet. 184, 394-399.

7. Hanahan, D. (1983) J.Mol.Biol. 166, 557-580.

8. Gautvik, K.M., Teig, V., Halvorsen, J.F., Arnesen, E., Myhre, L., Heimann, P., and Tollman, R. (1979) Scand.J.clin.Lab.Invest. 39, 469-478.

9. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular cloning (a laboratory manual), Cold Spring Harbor Laboratory, New York.

10. Hendy, G.N., Kronenberg, H.M., Potts, J.T.Jr., and Rich, A. (1981) Proc.Natl.Acad.Sci. 78, 7365-7369.

11. Maxam, A., and Gilbert, W. (1980) Meth.Enzymol. 65, 499.

12. Chen, E.Y, and Seeburg, P.H. (1985) DNA 4, 165-170.

13. Laemmli, U.K. (1970) Nature 227, 680-685.

14. Aurbach, G.D. (1959) J.Biol.Chem. 234, 3179-3181.

15. Matsudaira, P. (1987) J.Biol.Chem. 262, 10035-10038.

16. Gautvik, K.M., Gordeladze, J.O., Jahnsen, T., Haug, E., Hansson, V., and Lystad, E. (1983) J.Biol.Chem. 258, 10304-10311.

17. Gautvik, K.M., Gordeladze, J.O., Moxheim, E., and Gautvik, V.T. (1984) Eur.Surg.Res. 16 (suppl.2), 41-54.

18. Brosius, J., and Holy, A. (1984) Proc.Natl.Acad.Sci. 81, 6929-6933.

19. de Boer, H.A., Comstock, L.J., and Vasser, M. (1983) Proc.Natl.Acad.Sci. 80, 21-25.

20. Brosius, J., Ullrich, A., Raker, M.A., Gray, A., Dull, T.J., Gutell, R.R., and Noller, H.F. (1981) Plasmid 6, 112-118.

21. Marston, F.A.O. (1986) Biochem.J. 240, 1-12.

22. Kronenberg, H.M., McDevitt, B.E., Majzoub, J.A., Nathans, J., Sharp, P.A., Potts, J.T.Jr., and Rich, A. (1979) Proc.Natl.Acad.Sci. 76, 4981-4985.

23. Breyel, E., Morelle, G., Auf'mkolk, B., Frank, R., Blöcker, H., and Mayer, H. (1984) Synthesis of mature human parathyroid hormone in Escherichia coli (Dechema, ed.), pp.363-369. Verlag Chemie, Weinheim.

24. Rabbani, S.A., Yasuda, T., Bennett, H.P.J., Sung, W.L., Zahab, D.M., Tam, C.S., Goltzman, D., and Hendy, G.N. (1988) J.Biol.Chem. 263, 1307-1313.

25. Wingender, E., Bercz, G., Blocker, H., Frank, R., and Mayer, H. (1989) J.Biol.Chem. 264, 4367-4373.

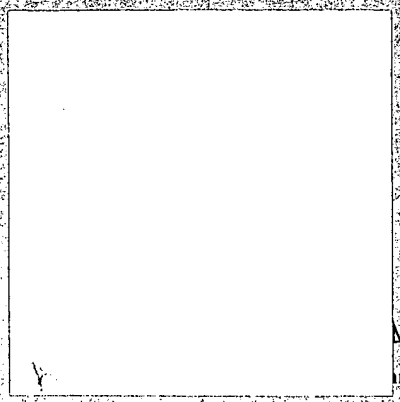
26. Born, W., Freeman, M., Hendy, G.N., Rapoport, A., Rich, A., Potts, J.T.Jr., and Kronenberg, H.M. (1987) Mol.Endocrinol. 1, 5-14.

27. Aurbach, G.D., Keutmann, H.T., Niall, H.D., Tregear, G.W., O'Riordan, J.L.H., Marcus, R., Marx, S.J., and Potts, J.T.Jr. (1972) Recent Prog.Horm.Res. 28, 353-398.

ISSN 0006-291X

Volume 166, Number 1, January 15, 1990
Pages 1–556

BIOCHEMICAL AND BIOPHYSICAL Research Communications



ACADEMIC PRESS, INC.
A Harcourt Brace Jovanovich, Publishers
San Diego New York Boston
London Sydney Tokyo Toronto

Paper No. 40

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte KAARE M. GAUTVIK, PETER ALESTROM, TORDIS B. OYEN and ODD S. GABRIELSEN

Appeal No. 2001-1974
Application No. 08/340,664

ON BRIEF

Before WINTERS, SCHEINER, and ADAMS, Administrative Patent Judges.

ADAMS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on the appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 31-35, which are all the claims pending in the application.

Claims 31 and 33 are illustrative of the subject matter on appeal and are reproduced below:

31. hPTH (1-84), as a substantially homogeneous protein.

33. hPTH (1-84), as a substantially homogeneous protein, prepared by a process comprising the steps of:

providing a microorganism containing exogenous DNA encoding hPTH (1-84);

culturing said microorganism to allow expression of said exogenous DNA, thereby producing hPTH (1-84); and

purifying said hPTH (1-84) as a substantially homogenous protein.

The references relied upon by the examiner are:

Brewer (Brewer)	3,888,132	May 7, 1995
Mayer (Mayer)	EP 0,139,076	May 2, 1985
Kaisha et al. (Kaisha)	GB 2,092,596 A	Aug. 18,1982

Breyel et al. (Breyel), "Synthesis of Mature Human Parathyroid Hormone in Escherichia coli," Third European Congress on Biotechnology, Vol. III, pp. 363-69 (1984)

Sung et al. (Sung), "Hybrid Gene Synthesis: its application to the assembly of DNA sequences encoding the human parathyroid hormones and analogues," Biochem. Cell Bio., Vol. 63, pp. 133-38 (1986)

GROUNDS OF REJECTION

Claims 33-35 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite.

Claims 31 and 32 stand rejected under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative, under U.S.C. 103 as obvious over Brewer.

Claims 31-34 stand rejected under 35 U.S.C. § 103 as being unpatentable over any one of Breyel, Sung, or Mayer, in view of Kaisha.

We affirm the rejection of claims 31 and 32 under 35 U.S.C. § 102(b) as being anticipated by Brewer. Accordingly, we do not reach the alternative rejection of claims 31 and 32 under 35 U.S.C. § 103 as being unpatentable over Brewer. We reverse the rejection of claims 33-35 under 35 U.S.C. § 112, second paragraph. We reverse the rejection of claims 31-34 under 35 U.S.C. § 103 as being unpatentable over either Breyel or Sung in view of Kaisha. We vacate the rejection of claims 31-34 under 35 U.S.C. § 103 over Mayer in view of

Kaisha. In addition, we identify other issues for the consideration of both the examiner and appellants.

CLAIM GROUPING

Appellants set forth two groupings of claims: (I) claims 31 and 32; and (II) claims 33-35. Brief, page 5. According to appellants the claims in each claim group stand or fall together. Id. Accordingly, we limit our discussion to representative independent claims 31 and 33. Claim 32 will stand or fall together with claim 31, and claims 34 and 35 will stand or fall together with claim 33. 37 CFR § 1.192(c)(7) (1998).

DISCUSSION

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH:

The examiner finds (Answer, page 4), "[t]he claims as they are currently written contain no reference to the secretory leader sequence and recite only expression of hPTH[ ](1-84), which is not described by the specification as originally filed." According to the examiner (id.), "[t]he omission of the sequence encoding the secretory leader amounts to a gap between the elements of the DNA to be expressed in the method recited in the claim, which is a product by process type claim."

We see no requirement in claims 33-35 that hPTH be secreted from the microorganism. Claim 33 simply requires hPTH be produced followed by the purification of hPTH as a substantially homogeneous protein. Claims 34 and 35 merely identify the microorganism as E. coli (claim 34) or yeast (claim 35). In addition, we cannot agree that the specification as "originally filed" contains no

reference to hPTH (1-84) as recited in appellants' claims. To the contrary, the specification discloses (page 5) that mature hPTH is an 84 amino acid protein. Furthermore, page 3 of the specification discloses, "it is an object of the present invention to provide a plasmid for insertion in yeast containing DNA coding for parathyroid hormone." As set forth in Amgen Inc. v. Chugai Pharmaceutical Co., Ltd., 927 F.2d 1200, 1217, 18 USPQ2d 1016, 1030 (Fed. Cir. 1991):

> The statute requires that "[t]he specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention." A decision as to whether a claim is invalid under this provision requires a determination whether those skilled in the art would understand what is claimed. See Shatterproof Glass Corp. v. Libbey-Owens Ford Co., 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir. 1985) (Claims must "reasonably apprise those skilled in the art" as to their scope and be "as precise as the subject matter permits.").

Furthermore, claim language must be analyzed "not in a vacuum, but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary skill in the pertinent art." In re Moore, 439 F.2d 1232, 1235, 169 USPQ 236, 238 (CCPA 1971). In this regard, we recognize appellants' argument (Brief, page 6) that the specification discloses, "a variety of leader sequences can be employed in the recombinant production of hPTH according to the invention. See page 5, lines 6-10, of the [a]pplication."

For the foregoing reasons, we disagree with the examiner that the claims contain a missing element and are therefore indefinite. Accordingly, we reverse the rejection of claims 33-35 under 35 U.S.C. § 112, second paragraph.

REJECTION UNDER 35 U.S.C. § 102(b)/103:

Claim 31 stands rejected under 35 U.S.C § 102(b) as anticipated by or, in the alternative, under 35 U.S.C § 103 as being obvious over Brewer. To support this rejection, the examiner finds (Answer, page 5), Brewer "disclose highly purified human PTH." According to the examiner (id.), Brewer's "preparation was pure enough to sequence 34 amino acid residues starting at the amino terminus of the protein. Thus, the protein as purified by Brewer et al. appears to be consistent with the limitations of the instant claims with respect to being 'substantially homogeneous' hPTH. Correlating Brewer to appellants' specification, the examiner finds (id.),

> the only portion of the specification which relates to purity is the disclosure that the protein was partially sequenced (page 7, starting at line 27), which the ordinary artisan would recognize as requiring a relatively pure preparation of the desired protein.... Based upon the fact that the specification discloses obtaining the sequence of 19 and 45 amino acids respectively, from the yeast and E. coli-produced protein, Brewer's ability to obtain 34 amino acids would seem to indicate that comparable purity was achieved.

In response, appellants note (Brief, page 7), "[h]omogeneity is not needed for sequencing." While this may be true, claim 31 is not drawn to a homogenous protein. Instead, claim 31 is drawn to a "substantially homogenous" protein. Since, homogenous would refer to a pure preparation, it is unclear from the specification what degree of purity is intended by "substantially" homogenous. We are unable to identify, and appellants do not refer to, a portion of appellants' specification that defines the term "substantially homogenous." Therefore, to the

extent that Brewer's purification protocol results in impure material[1], appellants have not established on this record that the impure material of claim 31 is different from that disclosed by Brewer.

We are not persuaded by appellants' argument that the hPTH sequence disclosed by Brewer contains errors. In this regard, we agree with the examiner (Answer, page 8) that appellants' claims are not limited to any particular sequence, and when read in light of the specification (e.g. page 15) may include non-native human sequences. We are also not persuaded by appellants' arguments (Brief, pages 8-9) regarding biological activity of hPTH, since claim 31 does not require the substantially homogeneous protein to be biologically active. See Answer, page 10.

To the extent that appellants argue (Brief, page 8) that Brewer does not disclose an intact protein, we note that Brewer disclose (column 1, lines 19-22), "[t]he complete amino acid sequence of the 84 amino acid parathyroid hormone from the bovine and porcine species have been reported." Brewer further disclose (column 2, lines 50-54), "[t]he purified human parathyroid hormone migrated as a single component on disc gel electrophoresis with a mobility which was identical to that of the bovine parathyroid hormone." This migration pattern suggests that Brewer's purified human parathyroid hormone is 84 amino acids in length.

Anticipation under 35 U.S.C. § 102 requires that a single prior art reference disclose each and every limitation of the claimed invention. Electro

[1] See appellants arguments (Brief, page 7) and paragraph 9 of the Maggio Declaration.

Med. Sys. S.A. v. Cooper Life Sci., 34 F.3d 1048, 1052, 32 USPQ2d 1017, 1019 (Fed. Cir. 1994). In our opinion the examiner has provided the evidence necessary to establish that Brewer discloses a substantially homogeneous hPTH (1-84) protein. Accordingly, we affirm the rejection of claim 31 under 35 U.S.C. § 102(b) as anticipated by Brewer. Having found that Brewer anticipates claim 31, we do not address the alternative rejection of claim 31 under 35 U.S.C. § 103. As set forth above, claim 32 falls together with claim 31.

REJECTION UNDER 35 U.S.C. § 103:

Claims 31-34 are rejected under 35 U.S.C. § 103 as being unpatentable over any one of Breyel, Sung, or Mayer in view of Kaisha.

Breyer or Sung in view of Kaisha:

According to the examiner (Answer, page 6) each of Breyel, Sung and Mayer teach the expression of recombinant hPTH in bacteria, but do not purify the protein from the bacterial cell extracts. The examiner relies on Kaisha to make up for this deficiency in Breyel, Sung and Mayer. According to the examiner (Answer, bridging paragraph, pages 6-7), Kaisha teaches that hPTH can be easily obtained by conventional purification and separation teachniques.

In response appellants argue (Brief, page 10) that Breyel teaches that PTH has a short half-life in E. coli, "[t]hus, from the moment the hPTH is produced by E. coli in the method of Breyel et al., the hPTH composition will be contaminated by PTH fragments produced by the degradation of PTH." Accordingly, appellants conclude (id.), "Breyel et al. do not teach an intact and substantially homogeneous hPTH protein, as claimed by [a]pplicants."

Regarding Sung, appellants argue (id.), "Sung et al. did not produce recombinant hPTH protein, instead they constructed a plasmid having the sequence for hPTH(1-84), and suggest that they are conducting a study of the expression of these gene products." In addition, appellants note (Brief, bridging paragraph, pages 10-11) that since Sung inserted the plasmids in an E. coli expression system they would be expected to have a short half-life as taught by Breyel. Accordingly, appellants conclude (Brief, page 11), "Sung et al. do not teach or suggest [a]pplicants' claimed invention."

Appellants' arguments (Brief, page 12) limit Kaisha to the purification of hPTH from human lymphoblastoid cells, which according to appellants are "barely contaminated with host animal cells." We are not persuaded by this argument. The fact that lymphoblastoid cells would be "barely contaminated with host animal cells" would be equally true for a pure culture of any cell, e.g. the bacteria taught by Breyel and Sung. Nevertheless, we are persuaded by appellants' arguments concerning Breyel and Sung.

According to the examiner (Answer, page 12), "[i]t would have been within the skill of the ordinary artisan to have separated the full-length (1-84) [hPTH protein] from the degraded forms of the product, to obtain the claimed homogeneous preparation." According to the examiner (id.), given the teachings of Kaisha, a "person of ordinary skill in the art would be able to devise a protocol for purifying such [a degraded preparation of protein] with a reasonable expectation of success and without undue experimentation...." However, we find nothing in Kaisha to support this position, and the examiner offers no additional

evidence to support her position that it would be within the skill of the art as taught by Kaisha to purify intact hPTH (1-84) from a degraded preparation protein with a reasonable expectation of success. As appellants argue, there is no suggestion in either of Breyel or Sung that any intact protein could be obtained. Based on the evidence of record, it is our opinion that, at best, it would have been obvious to try to isolate "intact protein" from the degraded preparations of Breyel and Sung. "Obvious-to-try," however, is not the standard of obviousness under 35 U.S.C. § 103. See In re O'Farrell, 858 F.2d 894, 903, 7 USPQ2d 1673, 1680 (Fed. Cir. 1988). Accordingly, we reverse the rejection of claims 31-34 under 35 U.S.C. § 103 as being unpatentable over either Breyel, or Sung, in view of Kaisha.

Mayer in view of Kaisha:

This rejection stands on a different footing. The Mayer document relied upon by the examiner is in the German language. This record does not reflect that the examiner considered an English language translation of this foreign language document. We recognize with appreciation that appellants included a copy of the English language abstract for this document. However, the evidentiary basis for this rejection is the full text of this document, and it stands to reason that full text documents will provide more facts. Since obviousness determinations are fact-intensive, it is not apparent why the examiner and appellants have satisfied themselves with determining patentability under 35 U.S.C. § 103 on less than a complete factual record.

As set forth in Gechter v. Davidson, 116 F.3d 1454, 1457, 43 USPQ2d 1030, 1033 (Fed. Cir. 1997), "For an appellate court to fulfill its role of judicial review it must have a clear understanding of the grounds for the decision being reviewed," which requires that "[n]ecessary findings must be expressed with sufficient particularity to enable [the] court without resort to speculation, to understand the reasoning of the board, and to determine whether it applied the law correctly and whether the evidence supported the underlying and ultimate fact-findings." Like the Court of Appeals in Gechter, this board requires a clear understanding of the grounds for the decision being reviewed. In this case, we find it difficult to understand the examiner's reasoning and whether the evidence upon which he relies supports the underlying fact-findings for the rejections under 35 U.S.C. § 103. The board cannot examine in the first instance all applications which come before it in an ex parte appeal under 35 U.S.C. § 134. In this particular appeal, we have elected not to expend board resources to obtain the needed translation. Accordingly, we vacate the rejection of claims 31-34 under 35 U.S.C. § 103 as being unpatentable over Mayer in view of Kaisha, and remand the application to the examiner for further consideration. Upon return of the application, the examiner should obtain an English language translation of the Mayer document. If upon review of this document, the examiner remains of the opinion that the claims on appeal are unpatentable, she should issue an appropriate Office Action that sets forth the facts and reasons used in support of such a rejection.

OTHER ISSUES

Product-by-process:

We note that claims 33-35 are drafted in product-by-process format. Similar to claim 31, claims 33-35 require only that the protein preparation be "substantially homogeneous." Therefore, absent evidence to the contrary, a reference that anticipates, or renders obvious claim 31, would also anticipate, or render obvious claims 33-35. We remind the examiner and appellants that the determination of patentability in product-by-process claims is based on product itself, even though such claims are limited and defined by a process. In re Thorpe, 777 F.2d 695, 697, 227 USPQ 964, 966 (Fed. Cir. 1985). "[W]here a product-by-process claim is rejected over a prior art product that appears to be identical, although produced by a different process, the burden is upon the applicants to come forward with evidence establishing an unobvious difference between the claimed product and the prior art product." In re Best, 562 F.2d 1252, 1255, 195 USPQ 430, 433 (CCPA 1977); In re Marosi, 710 F.2d 799, 803, 218 USPQ 289, 292-93 (Fed. Cir. 1983).

Accordingly, we encourage the examiner to take a step back and reconsider claims 33-35 together with the available prior art. If after this review the examiner finds that the claims on appeal are unpatentable, she should issue an appropriate Office Action that sets forth the facts and reasons used in support of such a rejection.

Standard:

We note that appellants' specification teaches (e.g., page 7) the use of an "hPTH standard(1-84)" to compare and assess the results of the purification process. The specification teaches that the purification product eluted in the same peak as this standard and comigrated with the standard as one band on a gel. The specification implicitly asserts that these comparisons demonstrate the purity and completeness of the protein being claimed. Upon return of the application, the examiner and appellants should work together to determine whether this "standard" is available prior art against claims 31-35.

105?

FURTHER PROCEEDINGS

We are not authorizing a Supplemental Examiner's Answer under the provisions of 37 CFR § 1.193(b)(1). Any further communication from the examiner that contains a rejection of the claims should provide appellants with a full and fair opportunity to respond.

TIME PERIOD FOR RESPONSE

In addition to affirming the examiner's rejection of one or more claims, this decision contains a remand to the examiner. Therefore, in order to preserve appellants' right to seek review under 35 U.S.C. § 141 or 145 with respect to the affirmed rejection, the effective date of the affirmance is deferred until conclusion of the prosecution before the examiner unless, as a mere incident to the limited prosecution, the affirmed rejection is overcome.

If further prosecution before the examiner does not result in allowance of the application, abandonment or a second appeal, this case should be returned

to the Board of Patent Appeals and Interferences for final action on the affirmed rejection, including any timely request for rehearing hereof.

No time period for taking subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

AFFIRMED-IN-PART; VACATED and REMANDED

Sherman D. Winters
Administrative Patent Judge

Toni R. Scheiner
Administrative Patent Judge

Donald E. Adams
Administrative Patent Judge

BOARD OF PATENT APPEALS AND INTERFERENCES

FOLEY & LARDNER
Suite 500
3000 K Street, N.W.
Washington, DC 20007-5109